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Phytanoyl-coenzymeA 2-hydroxylase

L.Odell, S. Gabelli, J. Morrell, S. Gould, and L. Amzel (Johns Hopkins U. School of Medicine)

Beamline(s): X25

Introduction: Phytanoyl-coenzymeA 2-hydroxylase (PAHX) is a non-heme Fe(II), 2-oxoglutarate (2-OG) dependent dioxygenase that catalyzes the 2-hydroxylation of phytanoyl-coenzymeA (-CoA) in mammals. The 2-hydroxylation of phytanoyl-CoA is the first step in the metabolism of phytanic acid, a branched chain isoprenoid fatty acid derived from chlorophyll. Loss of PAHX activity and the associated increase in phytanic acid characterize the disorder Refsum's disease. We would like to determine PAHX's atomic resolution structure to learn more about its enzymatic mechanism and its role in Refsum's disease. PAHX has no known structural homologues and, thus, requires ab initio phase determination. Attempts at MIR have been thwarted by nonisomorphism and lack of apparent derivatization.

Methods and Materials: On this run, a preliminary Hg SAD experiment was performed on PAHX 2-OG, O_2 co-crystals. The crystals are 0.1 x 0.06 x 0.04 mm and belong to space group P21 with the unit cell dimensions a=45.1 b=82.8 c=78.6 α=90.0 β=91.8 γ=90.0. Crystals were soaked for two hours in 0.1 mM mercuric acetate. An EXAFS scan of these crystals was not performed, but a dataset was collected using an X-ray wavelength, 1.0000 Å, close to mercury's anomalous edge, 1.0093 Å. Data were collected to 1.8 Å resolution on a CCD detector (ADSC Q315).

Results: Diffraction data, processed with Denzo and Scalepack, have an R_{sym} of 6.6% and extend to 1.8 Å resolution. The dataset yielded a small anomalous signal, insufficient to use SAD to solve for phases. The data scaled well to native data collected using CuK_α radiation from a rotating anode. An isomorphous difference Patterson map was constructed, and the anomalous signal aided in the interpretation of the map. Two Hg sites were identified using CNS that resulted in a figure of merit of 0.14 upon phasing.

Conclusions: Two sites were identified for Hg derivatized PAHX crystals, however, the occupancy of each site was too low to yield useful phases. If crystals can withstand to be soaked longer or in a higher concentration of mercuric acetate, we will achieve higher occupancy of the identified sites, as well as potential additional sites. The crystals should then yield a stronger anomalous signal and will be suitable for using Hg SAD/MAD to solve for the phases.

References: A.S. Wierzbicki, M.D. Lloyd, C.J. Schofield, M.D. Feher, and F.B. Gibberd, "Refsum's disease: a peroxisomal disorder affecting phytanic acid α-oxidation, " Journal of Neurochemistry, **80**, 727-735.